Amendments to the Specification:

Please replace paragraph 56 with the following new paragraph.

[0056] Arginine deiminase catalyzes the conversion of arginine to citrulline, and may be used to eliminate arginine. In the present invention, the arginine deiminase gene may be derived, cloned or produced from any source, including, for example, microorganisms, recombinant biotechnology or any combination thereof. Arginine deiminase may be cloned from microorganisms of the genus *Mycoplasma*. In some embodiments, the arginine deiminase is cloned from *Mycoplasma arginini, Mycoplasma* ~~*hominus*~~ *hominis, Mycoplasma* ~~*arthritides*~~ *arthritidis*, or any combination thereof. In some embodiments, the arginine deiminase used in the present invention may have one or more of the amino acid sequences of SEQ ID NOS: 1-10 and 13-21.

Please replace paragraph 60 with the following new paragraph.

[0060] Chemical and genetic modification of the arginine deiminase enzyme can affect its biological activities. For example, it has been shown that arginine deiminase is typically antigenic and rapidly cleared from circulation in a patient. However, it has also been shown that the formulation of arginine deiminase with polyethylene glycol reduces the antigenicity and increases the circulating half-life of the enzyme. Abuchowski et al., *Cancer Biochem. Biophys.* 7:175-186 (1984); Abuchowski et al., *J. Biol. Chem.* 252:3582-3586 (1977). In particular, arginine deiminase can be covalently modified with polyethylene glycol. Arginine deiminase covalently modified with polyethylene glycol (with or without a linking group) may be hereinafter referred to as "ADI-PEG." In U.S. Patent Application Serial No. 09/023,809, Clark describes improved modifications of arginine deiminase from *Mycoplasma* ~~*hominus*~~ *hominis* (SEQ ID NO:1), *Mycoplasma arginini* (SEQ ID NO:5), and *Mycoplasma* ~~*arthritides*~~ *arthritidis* (SEQ ID NO:7) with polyethylene glycol, the disclosure of which is hereby incorporated by reference herein in its entirety. When compared to native arginine deiminase, ADI-PEG retains most of its enzymatic activity, is far less antigenic, has a greatly extended circulating half-life, and is much more efficacious in the treatment of tumors. For purposes of the invention, the modification of any arginine deiminase with polyethylene glycol may be referred to as pegylation.

Please replace paragraph 61 with the following new paragraph.

[0061] It is to be understood that arginine deiminase derived from other organisms may also have pegylation sites corresponding to 112 position of arginine deiminase from *Mycoplasma ~~hominus~~ hominis*. For example, arginine deiminase from *Streptococcus pyrogenes* has lysine at the 104 position, arginine deiminase from *Mycoplasma pneumoniae* has lysine at the 106 position, and arginine deiminase from *~~Qiardia~~ Giardia intestinalis* has lysine at the 114 position. In addition, arginine deiminase from some organisms may have lysines corresponding to the same general location as the 112 position of arginine deiminase from *Mycoplasma hominus*. The location of lysine in arginine deiminase from such organisms may be indicated as follows:

Table 1: Pegylation sites of arginine deiminase from various organisms

Organisms producing arginine deiminase	**Position of lysine in arginine deiminase**
Mycoplasma ~~hominus~~ hominis (SEQ ID NO:1)	112
Mycoplasma arginini (SEQ ID NO:5)	111
Clostridium perfringens(SEQ ID NO:18)	105
Bacillus licheniformis(SEQ ID NO:19)	97, 108
Borrelia burgdorferi(SEQ ID NO:15)	102, 111
Borrelia ~~afzellii~~ afzelii (SEQ ID NO:16)	101
Enterococcus faecalis(SEQ ID NO:20)	102, 110
Streptococcus pyogenes(SEQ ID NO:13)	104
Streptococcus pneumoniae(SEQ ID NO:14)	103
Lactobacillus ~~sake~~ sakei (SEQ ID NO:21)	97, 106
~~Qiardia~~ Giardia intestinalis(SEQ ID NO:17)	114, 116

Please replace paragraph 63 with the following new paragraph.

[0063] In some embodiments the present invention provides for certain amino acid substitutions in the polypeptide chain of arginine deiminase. These amino acid substitutions provide for modified arginine deiminase that loses less activity upon pegylation; i.e. upon

pegylation, the reduction of enzyme activity following pegylation in the modified arginine deiminases is less than the reduction of enzyme activity following pegylation in the unmodified arginine deiminases. By eliminating pegylation sites at or adjacent to the catalytic region of enzyme, optimal pegylation can be achieved without the traditional loss of activity. As discussed above, arginine deiminase from certain organisms have pegylation sites located at various positions on the peptide chain. While not limiting the present invention, it is presently believed that arginine deiminase may have the amino acid lysine located at or adjacent to the catalytic region of the enzyme and that pegylation of these sites may inactivate the enzyme. By eliminating at least one of these pegylation sites, pegylation can be achieved and more enzyme activity retained. In accordance with the invention, in some embodiments lysine is substituted with glutamic acid, valine, aspartic acid, alanine, isoleucine, leucine or combinations thereof. In some embodiments lysine is substituted with glutamic acid. In some embodiments of the invention, modified arginine deiminase from *Mycoplasma ~~hominus~~ hominis* has an amino acid substitution at Lys^{112}, Lys^{374}, Lys^{405}, Lys^{408} or combinations or subcombinations thereof. In some embodiments modified arginine deiminase from *Mycoplasma ~~hominus~~ hominis* has an amino acid substitution Lys^{112} to Glu^{112}, Lys^{374} to Glu^{374}, Lys^{405} to Glu^{405}, Lys^{408} to Glu^{408} or combinations thereof. In some embodiments modified arginine deiminase from *Mycoplasma ~~hominus~~ hominis* has lysine at position 112 substituted with glutamic acid (SEQ ID NO:2).

Please replace paragraph 64 with the following new paragraph.

[0064] The present invention thus provides for certain amino acid substitutions in the polypeptide chain of arginine deiminase. Such amino acid substitutions can eliminate the problematic structural characteristics in the peptide chain of arginine deiminase. Such amino acid substitutions provide for improved renaturation of the modified arginine deiminase. These amino acid substitutions make possible rapid renaturing of modified arginine deiminase using reduced amounts of buffer. These amino acid substitutions may also provide for increased yields of renatured modified arginine deiminase. In some embodiments of the invention, the modified arginine deiminase has a single amino acid substitution at Pro^{210}. As mentioned above, arginine deiminase derived from *Mycoplasma ~~hominus~~ hominis* has the

amino acid proline located at the 210 position. While not limiting the present invention, it is presently believed that the presence of the amino acid proline at position 210 results in a bend or kink in the normal polypeptide chain that increases the difficulty of renaturing (i.e., refolding) arginine deiminase. Substitutions for proline at position 210 may make possible the rapid renaturation of modified arginine deiminase using reduced amounts of buffer. Substitutions for proline at position 210 may also provide for increased yields of renatured modified arginine deiminase. In some embodiments, the proline at position 210 is substituted with serine (SEQ ID NO:3). It is to be understood that in accordance with this aspect of the invention, other substitutions at position 210 may be made. Examples of substitutions include Pro^{210} to Thr^{210}, Pro^{210} to Arg^{210}, Pro^{210} to Asn^{210}, Pro^{210} to Gln^{210} or Pro^{210} to Met^{210}. By eliminating those structural characteristics associated with the amino acid of position 210 of the wild-type arginine deiminase, proper refolding of the enzyme can be achieved.

Please replace paragraph 65 with the following new paragraph.

[0065] In some embodiments of the invention, the modified arginine deiminase has multiple amino acid substitutions. The modified arginine deiminase may have at least one amino acid substitution eliminating pegylation sites at or adjacent a catalytic region of the enzyme. The modified arginine deiminase may also have at least one amino acid substitution eliminating those structural characteristics that interfere with the renaturation of the enzyme. The amino acid substitutions may thus provide for a modified arginine deiminase of the invention. The amino acid substitutions may provide for the pegylation of modified arginine deiminase without a loss of enzymatic activity. The amino acid substitutions may provide for a modified arginine deiminase that can be rapidly renatured using reduced amounts of buffer. The amino acid substitutions may also provide for increased yields of renatured modified arginine deiminase. In some embodiments, the modified arginine deiminase derived from *Mycoplasma* ~~*hominus*~~ *hominis* includes the proline at position 210 substituted with serine and the lysine at position 112 substituted with glutamic acid (SEQ ID NO:4). As discussed above, however, it is to be understood that the modified arginine deiminase may include other substitutions. In some embodiments, conservative substitutions may be made at positions

112 and/or 210 of the wild-type arginine deiminase.

Please replace paragraph 66 with the following new paragraph.

[0066] Modified arginine deiminase was expressed in JM101 cells as previously described by Takaku et al., *supra*. The modified arginine deiminase included glutamic acid at the 112 position and serine at the 210 position. In some embodiments the amino acid sequence of modified arginine deiminase from *Mycoplasma ~~hominus~~ hominis* is a sequence of SEQ ID NO:4.

Please replace paragraph 67 with the following new paragraph.

[0067] In some embodiments arginine deiminase is derived from *Mycoplasma ~~hominus~~ hominis*, *Mycoplasma pneumoniae*, *Mycoplasma arginini*, *~~Qiardia~~ Giardia intestinalis*, *Clostridium perfringens*, *Bacillus licheniformis*, *Borrelia burgdorferi*, *Borrelia ~~afzellii~~ afzelii*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Lactobacillus ~~sake~~ sakei* or *~~Qiardia~~ Giardia intestinalis* arginine deiminase.

Please replace paragraph 68 with the following new paragraph.

[0068] In some embodiments arginine deiminase is derived from *Mycoplasma ~~hominus~~ hominis* arginine deiminase (SEQ ID NO:1). In some embodiments, the arginine deiminase comprises at the substitution or deletion of at least one proline residue as compared to SEQ ID NO:1. In some embodiments, the substitution or deletion of at least one proline residue comprises substitution or deletion of the proline residue at or corresponding to residue 210 of SEQ ID NO:1. In some embodiments, the substitution or deletion of at least one proline residue comprises substitution of the proline residue at or corresponding to residue 210 of SEQ ID NO:1 with Ser, Thr, Arg, Asn, Gln, or Met. In some embodiments, the substitution or deletion of at least one proline residue comprises substitution of the proline residue at or corresponding to residue 210 of SEQ ID NO:1 with Ser.

Please replace paragraph 72 with the following new paragraph.

[0072] In some embodiments the arginine deiminase from *Mycoplasma ~~hominus~~ hominis* comprises a substitution of lysine at residue 112 of SEQ ID NO:1 with glutamic acid (SEQ

ID NO:2). In some embodiments the arginine deiminase from *Mycoplasma ~~hominus~~ hominis* comprises a substitution of proline at residue 210 of SEQ ID NO:1 with serine (SEQ ID NO:3). In some embodiments the arginine deiminase from *Mycoplasma ~~hominus~~ hominis* comprises a substitution of lysine at residue 112 of SEQ ID NO:1 with glutamic acid and a substitution of proline at residue 210 of SEQ ID NO:1 with serine (SEQ ID NO:4). In some embodiments arginine deiminase from *Mycoplasma arginini* comprises a substitution of lysine at residue 111 of SEQ ID NO:5 with glutamic acid (SEQ ID NO:6). In some embodiments the arginine deiminase from *Mycoplasma ~~arthritides~~ arthritidis* comprises substitutions of lysine at residues 111 and 112 of SEQ ID NO:7 with glutamic acid (SEQ ID NO:8). In some embodiments the arginine deiminase from *Mycoplasma ~~arthritides~~ arthritidis* comprises a substitution of lysine at residue 111 of SEQ ID NO:7 with glutamic acid (SEQ ID NO:9). In some embodiments the arginine deiminase from *Mycoplasma ~~arthritides~~ arthritidis* comprises a substitution of lysine at residue 112 of SEQ ID NO:7 with glutamic acid (SEQ ID NO:10).

Please replace paragraph 85 with the following new paragraph.

[0085] Generally, PEG is attached to a primary amine of ADI. Selection of the attachment site of polyethylene glycol on the arginine deiminase is determined by the role of each of the sites within the active domain of the protein, as would be known to the skilled artisan. PEG may be attached to the primary amines of arginine deiminase without substantial loss of enzymatic activity. For example, ADI cloned from *Mycoplasma arginini*, *Mycoplasma ~~arthritides~~ arthritidis* and *Mycoplasma ~~hominus~~ hominis* has about 17 lysines that may be modified by this procedure. In other words, the 17 lysines are all possible points at which ADI can be attached to PEG via a biocompatible linking group, such as SS, SPA, SCM, SSA and/or NHS. PEG may also be attached to other sites on ADI, as would be apparent to one skilled in the art in view of the present disclosure.

Please replace paragraph 90 with the following new paragraph.

[0090] In some embodiments, the present invention provides methods of inhibiting viral replication in an individual comprising administering to said individual a therapeutically or prophylactically effective amount of a compound comprising ADI covalently bonded via a

linking group to polyethylene glycol, wherein each polyethylene glycol molecule has an average molecular weight of from about 10,000 to about 30,000. In some embodiments ADI is modified with polyethylene glycol molecules, each molecule having an average molecular weight of about 20,000. In some embodiments the linking group is selected from the group consisting of a succinimide group, an amide group, an imide group, a carbamate group, an ester group, an epoxy group, a carboxyl group, a hydroxyl group, a carbohydrate, a tyrosine group, a cysteine group, a histidine group and combinations thereof. In some embodiments the linking group is succinimidyl succinate. In some embodiments from about 7 to about 15 polyethylene glycol molecules are bonded to arginine deiminase. In some embodiments from about 9 to about 12 polyethylene glycol molecules are bonded to arginine deiminase. In some embodiments the arginine deiminase is derived from a microorganism of the genus *Mycoplasma*. In some embodiments the arginine deiminase is derived from *Mycoplasma arginini*, *Mycoplasma* ~~*hominus*~~ *hominis*, *Mycoplasma* ~~*arthritides*~~ *arthritidis* and combinations thereof. In some embodiments the virus is HCV. In some embodiments the methods further comprise the step of administering a therapeutically effective amount of an additional anti-viral agent prior to, simultaneously with, or following administration of the arginine deiminase.

Please replace paragraph 122 with the following new paragraph.

[00122] Cultures of *Mycoplasma arginini* (ATCC 23243), *Mycoplasma* ~~*hominus*~~ *hominis* (ATCC 23114) and *Mycoplasma* ~~*arthritides*~~ *arthritidis* (ATCC 23192) were obtained from the American Type Culture Collection, Rockville, Maryland.

Please replace paragraph 123 with the following new paragraph.

[00123] Arginine deiminase was cloned from *Mycoplasma arginini*, *Mycoplasma* ~~*hominus*~~ *hominis* and *Mycoplasma* ~~*arthritides*~~ *arthritidis* and expressed in *E. coli* as previously described by S. Misawa et al, J. Biotechnology, 36:145-155 (1994), the disclosure of which is hereby incorporated herein by reference in its entirety. Characterization, by methods known to those skilled in the art, of each of the proteins with respect to specific enzyme activity, K_m, V_{max} and pH optima revealed that they were biochemically indistinguishable from each other. The pH optima was determined using a citrate buffer (pH 5-6.5), a phosphate buffer (pH 6.5-

7.5) and a borate buffer (pH 7.5-8.5). The K_m and V_{max} were determined by incubating the enzyme with various concentrations of arginine and quantifying citrulline production. The K_m for the various enzymes was about 0.02 to 0.06 μM and the V_{max} was about 15-20 μmol/min/mg, the values of which are within standard error of each other.